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CHARACTERIZATION OF FACTORS MEDIATING OVIPOSITION SITE CHOICE BY *CULEX TARSALIS*

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ABSTRACT. Fermented infusions of organic matter were tested for their effects on *Culex tarsalis* oviposition. Bermuda grass infusion and polluted water collected from a natural oviposition site (La Brea tar pits, CA) enhanced oviposition rates, but an alfalfa infusion and water from a 2nd natural oviposition site (Prado Basin, CA) did not. Bermuda grass infusion was fractionated by dialysis and filter sterilization. Crude Bermuda grass infusion, and fractions of the infusion containing large molecules (>12,000 daltons), particulates, and microorganisms significantly increased oviposition rates compared to distilled water controls. The fraction containing small molecules was no better than a distilled water control, suggesting that small molecules are not involved in oviposition stimulation in this species. However, using the egg raft counting bioassay, the possibility that the small molecules fraction contained oviposition attractants could not be ruled out. Overall, our experiments suggest that results obtained with the egg raft counting bioassay, which has been used frequently to screen for oviposition attractants, should be interpreted with caution. High oviposition rates in this bioassay may be due to responses to factors such as nonvolatile, contact oviposition stimulants rather than to volatile attractants.

INTRODUCTION

Oviposition site selection by a gravid female mosquito is mediated by a complex set of environmental factors, including chemical, physical, and biological stimuli in and around a particular site (reviewed by Bentley and Day 1989). It is not generally appreciated that mosquito oviposition behavior probably consists of a series of overlapping behavioral steps, each of which may be mediated by its own set of cues. These steps may include activation to begin flight, upwind flight towards an oviposition site, landing and sampling the site, and finally, egg deposition. Furthermore, considerable confusion has arisen with regard to the terms "attraction" and "stimulation" in the context of cues mediating mosquito oviposition (Benzon and Apperson 1988). Chemical cues mediating attraction towards a stimulus source must be volatile in order to act over a distance, whereas contact chemical stimuli mediating behaviors such as stimulation to deposit eggs can be either volatile or nonvolatile.

Mosquito oviposition attractants and stimulants consisting of infusions of fermented organic matter have been used extensively in surveillance programs aimed at monitoring both the size of mosquito populations and the presence of arboviruses in those populations (e.g., Fay and Eliason 1966; Loor and DeFoliart 1969; Madder et al. 1980; Leiser and Beier 1982; Reiter 1983, 1986, 1987). Gravid female traps baited with oviposition attractants are advantageous because they primarily collect mosquitoes that have bloodfed, and consequently may have become infected with an arbovirus. In contrast, CO_2 and light traps collect mainly uninfected, host-seeking females (Morris and DeFoliart 1971, Magnarelli 1975). The probability of detecting viral infections in a mosquito population is greatly increased by selectively sampling the bloodfed segment of the population (Surgeoner and Helson 1978).

Culex tarsalis Coq. is known to transmit viruses of concern to human and animal health (Western equine encephalitis and St. Louis encephalitis), and this species is a major target of surveillance efforts. Various natural or man-made infusions of organic matter have been shown to affect *Cx. tarsalis* oviposition. *Culex tarsalis* oviposition rates are enhanced by infusions of sod (Brust 1990) and steer manure (Reisen and Meyer 1990), but gravid females are repelled or deterred from ovipositing by a variety of other infusions, such as leaf litter (Reisen and Meyer 1990), chicken manure, and lab animal chow (Kramer and Mulla 1979). Furthermore, a recent field study determined that 3-methylindole, a compound known to enhance *Culex quinquefasciatus* Say oviposition, has a similar effect on gravid *Cx. tarsalis* (Beehler et al. 1994).

The objectives of the research described here were: 1) to identify organic infusions or natural waters that were attractive or stimulatory to gravid female *Cx. tarsalis,* and 2) to fractionate infusions and characterize the components in fractions that influenced mosquito oviposition.

MATERIALS AND METHODS

Mosquito colony: The *Cx. tarsalis* colony was started in November 1991 with larvae reared from egg rafts collected from the University of

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California, Riverside, Aquatic and Vector Control Facility at Oasis (Riverside Co.). The colony was augmented with egg rafts collected in the same locality in March 1992.

Larvae were reared at 25 ± 2°C, under a photoperiod of 12:10 L:D (120-W fluorescent light) with 1-h dusk and dawn periods provided by a 60-W incandescent light controlled by a timer. Larvae were reared in enamel pans (40 × 30 × 10 cm, approx. 1,000 larvae/pan) in 3 liters of distilled water. Larvae were fed *ad libitum* with a 3:1 mixture of ground dog chow and brewer's yeast.

Pupae were removed daily and transferred to glass jars (6 cm diam, 250 pupae/jar). Emerging adults were collected in cylindrical 2-liter cardboard containers with transparent tops placed over each pupal holding jar.

Adult *Cx. tarsalis* were maintained at 26 ± 1°C and 50 ± 10% RH, with L:D 12:10 (120-W fluorescent light) and 1-h dusk and dawn simulation photoperiods (60-W incandescent bulb). Newly emerged adults were held in cohorts in 2-liter cardboard containers for 3–4 days, then transferred to screen cages (45 × 45 × 45 cm) for 3 days to allow mating (1,000 individuals/cage). Mosquitoes were provided with raisins and 10% sugar water solution.

Adults were starved 24 h, then bloodfed overnight on 1–2-wk-old white leghorn chicks restrained in screen cylinders (UCR animal use protocol #A-93101-65). The following morning, bloodfed females were collected by aspiration and transferred to 2-liter containers (100 gravid females/container, plus 20 males to allow additional mating). Bloodfed mosquitoes were provided with raisins and sugar water.

Oviposition bioassays: Bioassays were conducted in a controlled environment chamber at 26 ± 1°C and 50 ± 10% RH, under a 12:10 L:D cycle as described above, and 1-h dusk and dawn periods. For the dusk period, the power to the 60-W bulb was decreased gradually over 1 h using a custom-built mechanical rheostat. For the dawn period the 60-W incandescent light was fully turned on 1 h before the fluorescent lights. Bioassays were conducted in rectangular cages (30 × 30 × 45 cm) made of a 1.25 cm polyvinyl chloride irrigation pipe frame covered in white gauze. Each cage was lined with white plastic-backed spill paper, which was replaced whenever solutions were spilled. Approximately 1 h prior to each bioassay, gravid females were aspirated into 400-ml containers (20 mosquitoes/container) and starved until bioassayed. Oviposition jars used in all bioassays were 6-cm-diam clear glass jars.

At 1700 h, oviposition jars containing 100 ml of test solution or distilled water were randomly placed 18 cm apart in each back corner of each bioassay cage. Gravid females were then introduced into each cage by placing the holding containers in the cages and removing the lids. The fluorescent lights were switched off at 1800 h, followed by the incandescent light gradually dimming to complete darkness at 1900 h. The next morning, the 60-W incandescent light was switched on at 0500 h, followed by the fluorescent lights at 0600 h. Bioassays were stopped at 0800 h and egg rafts were counted. In all bioassays, gravid females were used 6–9 days after a blood meal. Depending on the availability of gravid mosquitoes, 4–8 cages (1 cage = 1 replicate) were used in each bioassay, with at least 8 replicates/experiment. In all experiments, gravid females were used once and discarded.

Relationship between number of egg rafts laid and interval after bloodfeeding: Prior to investigating chemically mediated oviposition responses, the optimum interval between bloodfeeding and when bloodfed mosquitoes were most likely to oviposit was investigated. Cohorts of gravid *Cx. tarsalis* held without access to an oviposition site for 3–19 days after bloodfeeding were allowed to oviposit overnight in distilled water. Eight replicates (20 females/replicate) were conducted for each test.

Natural breeding water sources: Water samples were taken from seasonally flooded ponds in the Prado Basin (Riverside Co., CA) where *Cx. tarsalis* breed in large numbers, and from La Brea tar pits (Los Angeles Co., CA). The tar pits water was chosen because large numbers of *Cx. tarsalis* breed at this site, and *Cx. tarsalis* is the dominant breeding species (J. Beehler, unpublished data).

Fermented organic infusions: Bermuda grass (BGI) and alfalfa infusions (AI) were prepared in separate 250-liter fiberglass tubs by mixing 27 g lactalbumen hydrolysate, 27 g brewer's yeast, 450 g dry Bermuda grass cuttings or alfalfa hay cubes, and 100 liters of irrigation water. The tubs were covered and kept outdoors in shade for 7–10 days, depending on the fermentation progress. After 7–10 days, the infusion was coarsely filtered through a mesh screen, and frozen.

Undiluted AI and BGI, and BGI diluted with distilled water (20, 10, 4, and 0.8% of crude BGI in distilled water) were tested against distilled water controls for oviposition responses by gravid female *Cx. tarsalis.*

Fractionation of Bermuda grass infusion by dialysis (Fig. 1): Frozen infusions were thawed, then centrifuged at 1,000 rpm for 30 min to remove large particulates. Supernatants were transferred to dialysis tubing and the pellets were discarded.

The dialysis tubing (Spectra/Por 4, 28.6 mm

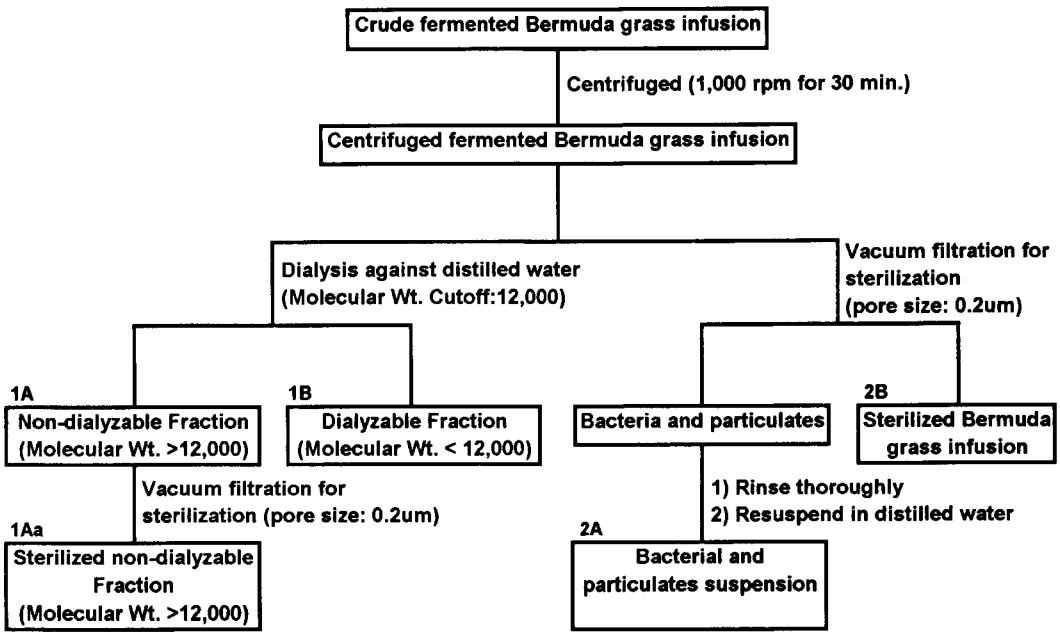

Fig. 1. Protocol for fractionation of Bermuda grass infusion.

diam and 45 mm long, 12,000–14,000 molecular weight [MW] cutoff; Fisher Scientific, Pittsburgh, PA) was prepared by rinsing with tap water for 4 h and distilled water for 2 h to remove glycol from the membrane. Each tube was loaded with 100 ml of centrifuged BGI and a glass marble (to weigh down tubes). Five tubes were processed in each lot. Tubing closures and glass marbles were autoclaved prior to use.

Five hundred milliliters of infusion were dialyzed at 4°C (to minimize metabolic activity by microorganisms) against 5 liters of distilled water in a covered glass battery jar with gentle stirring (magnetic stirrer). After 24 h, the dialysis water (Fig. 1, 1B), which now theoretically contained 90% of the compounds with less than 12,000–14,000 MW, was transferred to glass bottles and frozen. The tubes were dialyzed against 5 liters of distilled water 3 more times, then the nondialyzable fraction (Fig. 1, 1A) was transferred to glass bottles and frozen.

Sterilization of nondialyzable fraction of the infusion: The nondialyzable residues containing large molecules (more than 12,000–14,000 MW) and microorganisms were filter sterilized (VacuCap, Product No. 4622, Gelman Sciences, Ann Arbor, MI; 0.2 μm pore size), drawing the solution through the filter with vacuum. Sterilized filtrates (Fig. 1, 1Aa) were stored frozen in glass bottles.

Sterilization of Bermuda grass infusion: Crude Bermuda grass infusion was centrifuged, filter sterilized as described above, and frozen.

Resuspension of microorganisms and particulates: Microorganisms and particulates trapped on the filter membrane were recovered by first rinsing the membrane thoroughly with distilled water (5 × 100 ml) to remove traces of infusion, pulling the rinse water through the membrane with vacuum, and then reconstituting the microorganisms fraction with distilled water (500 ml). The reconstituted suspensions (Fig. 1, 2A) were frozen in glass bottles.

The dialyzable (1B) and nondialyzable (1A) fractions, and the sterilized nondialyzable fraction (1Aa) of the fermented Bermuda grass infusions, sterilized infusion (2B), and suspensions of bacteria and particulates (2A) were tested against distilled water and/or against each other for oviposition responses by gravid *Cx. tarsalis.* All samples were thawed at room temperature immediately prior to bioassay.

Oviposition response of Cx. tarsalis *to volatiles from BGI:* Two sets of bioassays were conducted on different days to determine whether volatiles from BGI enhanced oviposition rates. On the first day, a stimulus jar containing BGI was placed in the middle of each bioassay cage and an oviposition jar containing distilled water was placed in the back of each cage. Screens (1 mm mesh size and 6 cm diam) were placed over each stimulus jar so that mosquitoes could not contact the test stimulus. Egg rafts deposited in the oviposition jars were counted at the end of the overnight bioassay period. The experiment was repeated on the 2nd day, substituting a screened

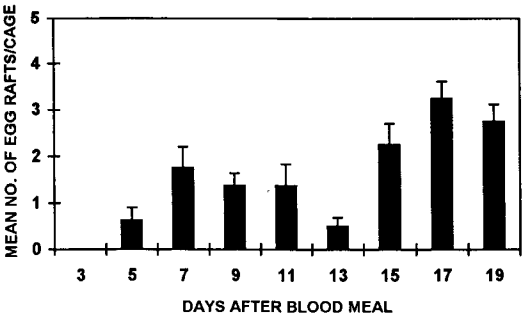

Fig. 2. Mean number (±SD) of egg rafts laid by gravid female *Culex tarsalis* as a function of days after blood meal. Eight replicates (20 females/replicate) were used.

jar of distilled water for the jar of BGI. Testing the BGI stimulus and the distilled water control on different days eliminated the possibility of BGI volatiles influencing oviposition in the cages containing controls.

Statistical analysis: Paired Student's *t*-tests compared differences between treatments and controls. Data in the study of the effect of volatiles from BGI were analyzed by unpaired *t*-tests.

RESULTS

Relationship between the number of egg rafts laid and days after bloodfeeding: To delineate optimal bioassay conditions, oviposition rates of bloodfed females at different intervals after bloodfeeding were determined, using distilled water as an oviposition medium (Fig. 2). Oviposition rates were low, probably due to the lack of oviposition stimulants in distilled water. Egg raft deposition was bimodal, with maxima between 7–10 days, and 15–19 days. Because few gravid females survived to 15 days, females between 6 and 10 days postbloodfeeding were used for all subsequent bioassays.

Chemical factors from natural breeding water and fermented organic infusions: Natural pond water and organic infusions were tested to determine their effects on *Cx. tarsalis* oviposition. Five concentrations of fermented Bermuda grass infusion (BGI) and water from the La Brea tar pits received more egg rafts than distilled water, whereas alfalfa hay infusion and Prado Basin pond water were no different than controls (Table 1). Even when diluted more than 100-fold, BGI received significantly more egg rafts than the control. Because BGI was readily available, all subsequent experiments were conducted with this infusion or fractions thereof.

Fractionation of Bermuda grass infusion (Fig. 1): The effect of chemical and biological factors in fractions of BGI on oviposition responses by *Cx. tarsalis* is summarized in Table 2. When tested against distilled water, the dialyzable fraction (Fig. 2, 1B) of BGI was no different than the control. When tested against equivalent concentrations of dilute BGI (10%), the dialyzable fraction (1B) received significantly fewer egg rafts than BGI.

Gravid females preferentially oviposited in jars containing the nondialyzable fraction (1A) of BGI when this fraction was tested against distilled water. Fraction 1A also received significantly more egg rafts than the dialyzable fraction (1B). There was no significant difference between the numbers of egg rafts deposited in fraction 1A and crude infusion.

The sterilized nondialyzable fraction (1Aa) of BGI was more stimulatory than the distilled water control (Table 2). However, the sterilized nondialyzable fraction (1Aa) was not significantly different than the dialyzable fraction (1B). Furthermore, when mosquitoes were given a choice between the sterilized nondialyzable fraction (1Aa) and BGI, mosquitoes deposited more egg rafts in BGI than in fraction 1Aa.

Further experiments were conducted with fil-

Table 1. Effect of various concentrations of Bermuda grass infusion, La Brea tar pits water, alfalfa infusion, and Prado Basin pond water on oviposition activity by *Culex tarsalis* gravid females.

Treatment	No. of egg rafts (mean ± SE)[1]	n^2
100% grass infusion	5.1 ± 0.7***	10
Distilled water	0.8 ± 0.4	
20% grass infusion	5.6 ± 1.1**	10
Distilled water	0.8 ± 0.3	
10% grass infusion	7.5 ± 0.8***	10
Distilled water	0.6 ± 0.2	
4% grass infusion	5.1 ± 0.8**	10
Distilled water	1.1 ± 0.6	
0.8% grass infusion	4.6 ± 0.6***	10
Distilled water	1.2 ± 0.3	
Tar pits water	3.9 ± 0.4**	12
Distilled water	1.6 ± 0.2	
Alfalfa infusion	2.2 ± 0.3[NS]	8
Distilled water	1.6 ± 0.2	
Prado pond water	1.9 ± 0.3[NS]	8
Distilled water	1.7 ± 0.3	

[1] Results analyzed by Student's *t*-test; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.

[2] Number of replicates. Each replicate consisted of 20 females.

Table 2. Comparison of the oviposition stimulatory activity of fractions of Bermuda grass infusion, with each other and with distilled water (neutral control) or crude infusion (positive control).

Treatment[1]	No. of egg rafts (mean ± SE)[2]	n[3]
Dialyzable fraction (1B)	2.8 ± 0.7[NS]	8
Distilled water	1.3 ± 0.5	
Nondialyzable fraction (1A)	5.5 ± 0.5***	8
Distilled water	0.9 ± 0.4	
Crude grass infusion	4.1 ± 0.6**	8
Dialyzable fraction (1B)	1.5 ± 0.3	
Crude grass infusion	3.6 ± 1.1[NS]	8
Nondialyzable fraction (1A)	3.4 ± 0.3	
Nondialyzable fraction (1A)	4.6 ± 0.8*	8
Dialyzable fraction (1B)	1.4 ± 0.4	
Sterilized nondialyzable fraction (1Aa)	3.3 ± 0.5***	8
Distilled water	1.2 ± 0.5	
Dialyzable fraction (1B)	3.0 ± 0.7[NS]	8
Sterilized nondialyzable fraction (1Aa)	2.5 ± 0.5	
Nondialyzable fraction (1A)	3.4 ± 0.9[NS]	8
Sterilized nondialyzable fraction (1Aa)	2.5 ± 0.5	
Crude grass infusion	9.1 ± 0.7***	8
Sterilized nondialyzable fraction (1Aa)	1.6 ± 0.2	

[1] Each treatment solution was 10% infusion equivalent.
[2] Paired Student's t-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant.
[3] Number of replicates. Each replicate consisted of 20 gravid females.

ter-sterilized infusion (2B) (Table 3). Females oviposited more in sterilized infusion than in distilled water. Sterilized infusion was also more stimulatory than the dialyzable fraction (1B) or the sterilized nondialyzable fraction (1Aa). Sterilization did result in the loss of some of the stimuli from crude BGI because gravid females laid fewer egg rafts in sterilized infusion (2B) than in crude infusion. However, there was no significant difference between sterilized crude infusion (2B) and the nonsterilized, nondialyzable fraction (1A).

Microorganisms and particulates trapped on the sterilizing filter and then resuspended in distilled water (fraction 2A) received more egg rafts than distilled water (Table 4). When a drop of black ink was added to both oviposition jars to enhance oviposition rates by providing visual cues, gravid females again oviposited more in fraction 2A than in distilled water. However, the suspension resulted in significantly less oviposition than crude infusion.

Oviposition response of Cx. tarsalis *to volatiles from BGI:* Mosquitoes exposed to volatile stimuli from BGI but prevented from contacting the stimulus source laid similar numbers of egg rafts in distilled water as mosquitoes not exposed to BGI volatiles (1.4 ± 1.2 egg rafts in each treatment; 8 replicates of 20 females/cage).

DISCUSSION

Under laboratory conditions, gravid *Cx. tarsalis* had high oviposition rates in crude or diluted Bermuda grass infusion (BGI) and in various fractions of BGI. Water taken from natural larval habitats in the Prado Basin in which wild *Cx. tarsalis* females were ovipositing extensively (as measured by the presence of egg rafts and immatures), was no more attractive or stimulatory than distilled water in laboratory bioassays. The lack of discrimination between distilled and Prado Basin water suggests that the latter does not contain significant amounts of oviposition stimuli. It also suggests that if no more suitable site is available, any still body of water may serve as an oviposition site for *Cx. tarsalis.*

The alfalfa infusion did not stimulate oviposition by *Cx. tarsalis,* despite being attractive to other *Culex* species (Reiter 1986, 1987). However, the effect of various infusions may be species dependent; an infusion that attracts one species may be inactive or repellent to others (Kramer

Table 3. Comparison of filter-sterilized Bermuda grass infusion with various fractions of the crude infusion.

Treatment[1]	No. of egg rafts (mean ± SE)[2]
Sterilized grass infusion (2B)	3.8 ± 0.5**
Distilled water	1.0 ± 0.4
Crude grass infusion	6.8 ± 0.6**
Sterilized grass infusion (2B)	3.8 ± 0.5
Sterilized grass infusion (2B)	4.4 ± 0.6*
Dialyzable fraction (1B)	2.4 ± 0.4
Sterilized grass infusion (2B)	4.6 ± 0.8[NS]
Nondialyzable fraction (1A)	2.0 ± 0.5
Sterilized grass infusion (2B)	3.9 ± 0.4*
Sterilized nondialyzable fraction (1Aa)	2.2 ± 0.4

[1] Each test solution was 10% infusion equivalent. Each experiment consisted of 8 replicates of 20 gravid females per replicate.
[2] Paired Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$; NS, not significant.

Table 4. Effect of bacterial suspension from Bermuda grass infusion on oviposition by *Culex tarsalis* females.

Treatment[1]	No. of egg rafts (mean ± SE)[2]	n[3]
Bacterial suspension (2A)	2.3 ± 0.9*	12
Distilled water	0.4 ± 0.3	
Bacterial suspension in dyed water (2A)	5.1 ± 0.6**	8
Dyed water	1.9 ± 0.6	
Grass infusion	5.0 ± 0.9***	8
Bacterial suspension (2A)	0.4 ± 0.5	

[1] Each test solution was 10% infusion equivalent.
[2] Paired Student's *t*-test; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.
[3] Number of replicates. Each replicate consisted of 20 gravid females.

and Mulla 1979), possibly reflecting interspecific differences in habitat selection cues.

Conversely, natural breeding water from the La Brea tar pits elicited significantly more oviposition than distilled water. The tar pits water had a distinctive, oily smell, but it was not clear whether the responses seen were due to volatile attractants or nonvolatile stimulants, or both.

The Bermuda grass infusion was chosen for further study because it was readily available, and could be reproduced as needed. In the first fractionation experiments, the dialyzable fraction containing small (volatile and nonvolatile) organic and inorganic compounds (Fig. 1, 1B) and a distilled water control received low and equal numbers of egg rafts. This result suggests that the small molecules in BGI are not oviposition stimulants for *Cx. tarsalis*. However, their role as potential oviposition attractants remains equivocal, because the egg raft counting bioassay may not evaluate attraction, despite the fact that it has often been used for this purpose. That is, gravid females attracted to the volatiles fraction may not oviposit in the absence of oviposition stimulants. Until there is a demonstrated correlation between attraction to an oviposition site (mediated by small volatile compounds) and actual egg deposition, results from the egg raft counting bioassay should be interpreted with caution. Large numbers of egg rafts laid in response to volatile stimuli indicate either attraction or oviposition stimulation or both, whereas few egg rafts laid indicate minimal oviposition stimulation, but no inferences can be made with regard to attraction. Consequently, the possibility that the dialyzable fraction (1B) contained oviposition attractants cannot be excluded.

Possible effects of volatile stimuli from BGI on mosquito oviposition were tested in a further experiment by exposing mosquitoes to BGI odors without allowing them to contact the stimulus source. We have observed that resting mosquitoes begin flying when BGI is introduced into a cage, even in daylight, but similar responses are not observed when distilled water is introduced. We hypothesized that the increase in flight activity in the presence of BGI volatiles might lead to an increase in oviposition rates due to more frequent encounters with oviposition cups containing distilled water. This was not the case; volatile stimuli from BGI in the cage atmosphere had no effect on oviposition rates.

More egg rafts were laid in the nondialyzable fraction (1A) containing large molecules, microorganisms, and particulates than in distilled water controls. Mosquitoes can only use contact chemoreceptors to assess this fraction because of the virtual absence of volatile materials (4 cycles of dialysis theoretically removed 99.99% of the volatiles). Furthermore, responses to this fraction were no different than responses to BGI. These results provide strong evidence that stimulation to oviposit is mediated primarily by large nonvolatile compounds, and suggest that the primary mode of action of BGI is oviposition stimulation rather than attraction.

However, fraction 1A also contained microorganisms whose metabolic products could be produced continuously during the bioassay; therefore, the effects of volatiles could not be completely discounted yet. To eliminate this possibility, the nondialyzable fraction (1A) was

filter sterilized. The resulting fraction 1Aa, containing negligible quantities of microorganisms or low molecular weight metabolites, resulted in oviposition rates equivalent to nonsterilized fraction (1A), indicating that the effects of metabolites produced during the bioassay were negligible.

To further clarify the roles of large molecules versus microorganisms, crude infusion was filter sterilized, and materials trapped on the filter were resuspended in distilled water. The filtrate (2B) contained all the components of BGI except particles larger than 0.2 μm in diameter. Both the filtrate (2B) and the resuspended material (2A) resulted in higher oviposition rates than the distilled water controls. More egg rafts were laid in crude infusion than sterilized infusion (2B), confirming that bacteria and/or particulates play an important role in mosquito oviposition, as suggested by a number of authors (Benzon and Apperson 1988, Bentley and Day 1989). Furthermore, the resuspended fraction (2A) was significantly less stimulatory than BGI, suggesting that responses to particulates and microorganisms are synergized by chemical components in BGI.

The mechanism by which mosquitoes detect microorganisms or particulate matter is not known. However, several *Culex* species imbibe water from oviposition sites before beginning to lay eggs (Weber and Tipping 1990, 1993), suggesting that chemo- or mechanoreceptors in the mouthparts may be used to detect microorganisms and particulates.

In summary, our results indicate that large nonvolatile components in BGI are a major and possibly predominant factor influencing *Cx. tarsalis* oviposition site selection. Our results also illustrate potential shortcomings of the egg raft counting bioassay if this assay is used to assess the attractiveness of various oviposition stimuli. For example, crude BGI elicited high oviposition rates in this bioassay, which might lead one to conclude that the infusion is attractive. However, further investigation revealed that fraction 1A, containing large molecules and negligible amounts of volatiles, had the same effect as crude BGI (Table 2), suggesting that the primary mode of action of BGI is oviposition stimulation rather than attraction. The fact that the volatiles fraction (1B) received similar numbers of egg rafts as the distilled water control neither proved nor disproved that this fraction contained attractants, because the bioassay evaluation criterion (number of egg rafts laid) may be unrelated to the attractiveness of the test solution. As pointed out previously in a study of *Aedes aegypti* (Linn.) oviposition, bioassays specifically designed to assay long-range attraction need to be developed, rather than attempting to infer whether a stimulus is attractive by assessing oviposition rates (Benzon and Apperson 1988).

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